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"Double Click" Reaction on 7-Deazaguanine DNA: Synthesis and Excimer Fluorescence of Nucleosides and Oligonucleotides with Branched Side Chains Decorated with Proximal Pyrenes

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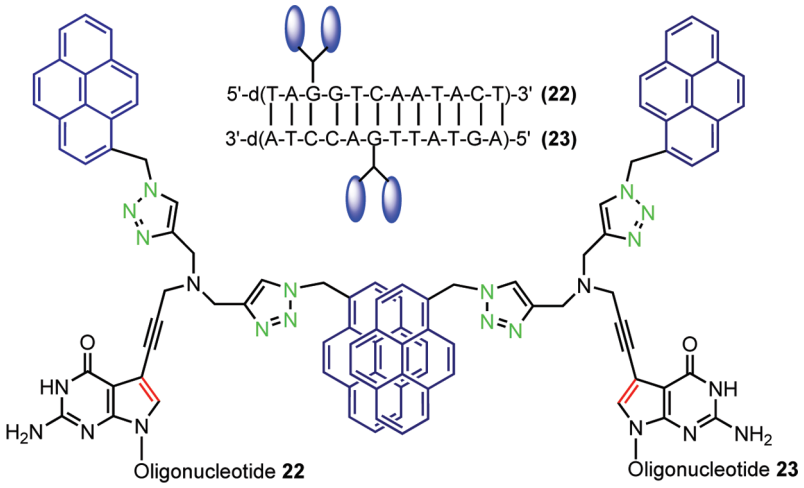
Received October 28, 2009



The 7-tripropargylamine-7-deaza-2′-deoxyguanosine (**2**) containing two terminal triple bonds in the side chain was synthesized by the Sonogashira cross-coupling reaction from the corresponding 7-iodo nucleoside **1b**. This was protected at the 2-amino group with an *iso*-butyryl residue, affording the protected intermediate **5**. Then, compound **5** was converted to the 5′-*O*-DMT derivative **6**, which on phosphitylation afforded the phosphoramidite **7**. This was employed in solid-phase synthesis of a series of oligonucleotides. T_m measurements demonstrate that a covalently attached tripropargylamine side chain increases duplex stability. Both terminal triple bonds of nucleoside **2** and corresponding oligonucleotides were functionalized by the Cu(I)-mediated 1,3-dipolar cycloaddition "double click reaction" with 1-azidomethyl pyrene **3**, decorating the side chain with two proximal pyrenes. While the monomeric tripropargylamine nucleoside with two proximal pyrenes (**4**) shows strong excimer fluorescence, the ss-oligonucleotide containing **4** does not. This was also observed for ds-oligonucleotides when the complementary strand was unmodified. However, duplex DNA bearing pyrene residues in both strands exhibits strong excimer fluorescence when each strand contains two pyrene residues linked to the tripropargylamine moiety. This pyrene−pyrene interstrand interaction occurs when the pyrene modification sites of the duplex are separated by two base pairs which bring the fluorescent dyes in a proximal position. Molecular modeling indicates that only two out of four pyrene residues are interacting forming the exciplex while the other two do not communicate.

Introduction

The Huisgen−Meldal−Sharpless cycloaddition "click" reaction performed with organic azides and alkynes has found broad application in chemistry,[1] biology,[2] drug development,[3] and material science.[4] The reaction is high yielding, robust, and can be performed under aqueous conditions.[1b] In the field of DNA and RNA chemistry as well as in chemical biology, the method has been used to introduce reporter groups into biomolecules in a very efficient way.[5] Recently,

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DOI: 10.1021/jo902300e

our laboratory has reported on nucleosides and oligonucleotides with alkynylated side chains in the 5-position of pyrimidines or the 7-position of 7-deazapurines and 8-aza-7-deazapurines.[6] The terminal triple bonds of the side chain were functionalized with various azides including those of AZT and coumarin.[6e,7] As there is an ongoing interest in the high density labeling of nucleosides and oligonucleotides, we have introduced a tripropargylamine residue instead of the octa-1,7-diynyl side chain in 2′-deoxyuridine as well as in corresponding DNA fragments.[8] Thus, a branched side chain was generated with two reactive terminal triple bonds. As two terminal triple bonds can be functionalized simultaneously, the density of labels on the oligonucleotide chain is increased.

The current method called "double click" reaction[8] brings the new ligands of the branched side chain in close proximity. This prompted us to study the functionalization of nucleosides and oligonucleotides with pyrene residues. Various pyrene derivatives of nucleosides and oligonucleotides have been prepared. This includes the use of pyrene as nucleobase surrogate[9] or conjugated to the sugar[10] or abasic site in DNA[11] or base moiety of nucleosides[12] and oligonucleotides. Bispyrene probes have been prepared[13] as well as click reactions with **3** and pyren-1-yl azide have been reported.[14] They were used for DNA and RNA sensing[15] or as nanomaterials.[16]

Pyrene shows monomer as well as excimer fluorescence.[17] Excimer fluorescence occurs when one pyrene molecule in the excited state forms a contact dimer with a second molecule in the ground state.[18] We have selected 7-deaza-2′-deoxyguanosine (**1a**)[19] (Figure 1) as 2′-deoxyguanosine surrogate for DNA modification as it exhibits extraordinary chemical, physical, and biological properties. Base pairing is limited to the Watson−Crick mode;[20] it can be functionalized with rather bulky substituents at the 7-position without perturbing the DNA duplex structure,[6c,d,21] and its triphosphate is well-accepted by DNA polymerases.[22] Furthermore, 2′,3′-dideoxy derivatives have been already tethered with fluorescent dyes[23] and are used as chain terminators in the Sanger dideoxy sequencing protocol and MALDI-TOF mass spectrometry.[24]

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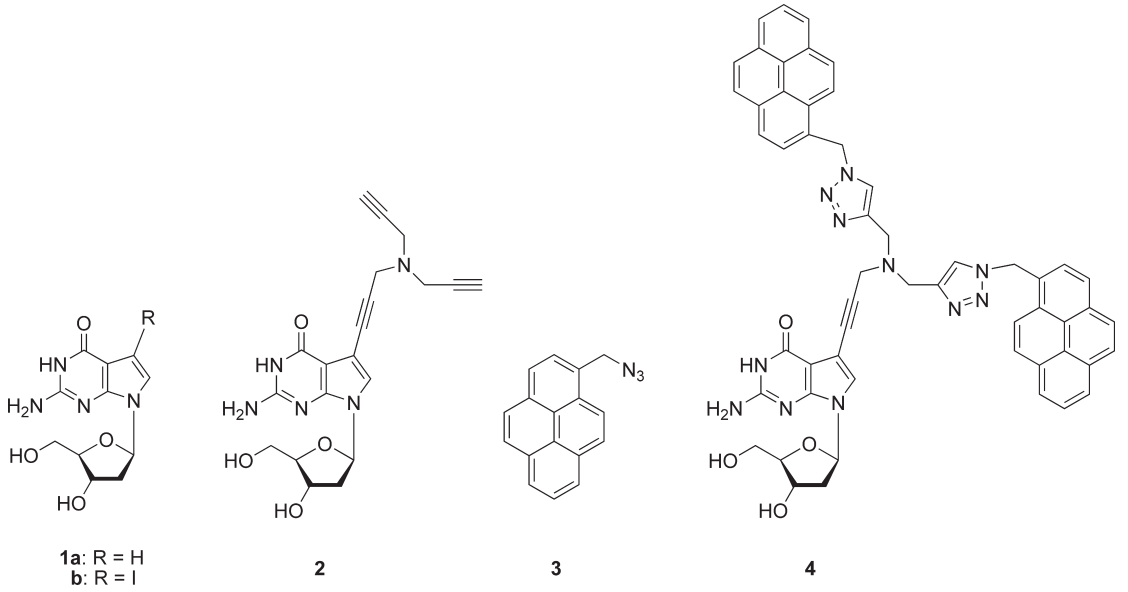
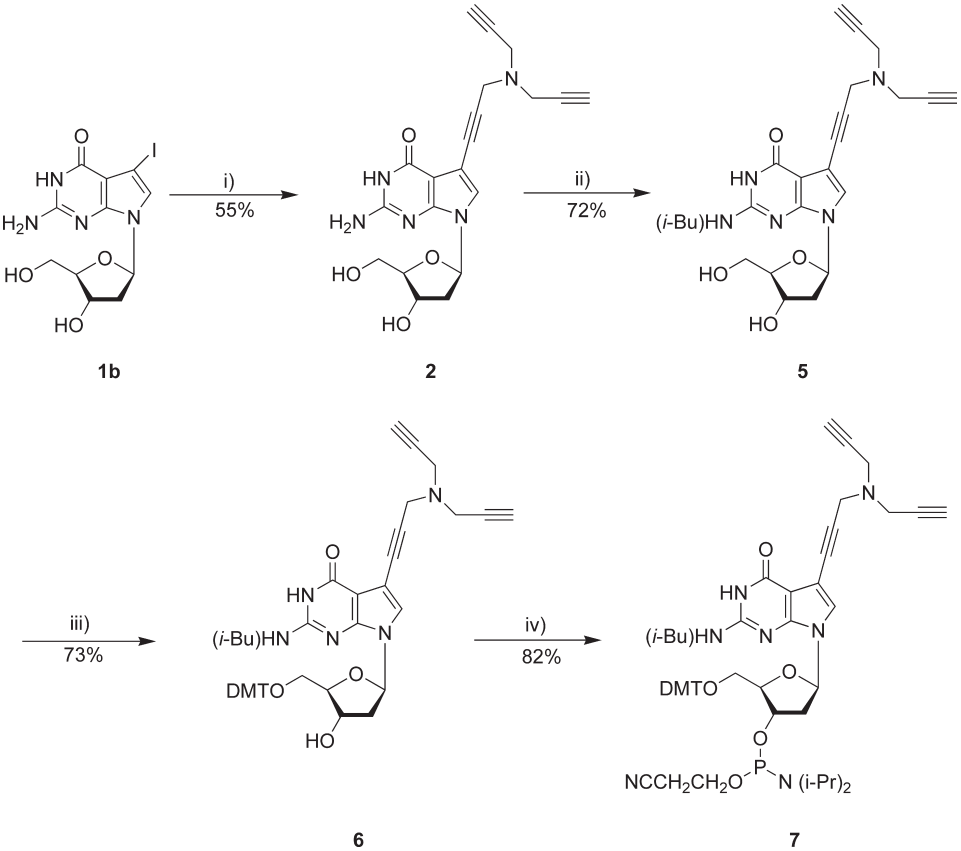


FIGURE 1. Structures of nucleosides, azide, and nucleoside click conjugate.

SCHEME 1. Synthesis of Phosphoramidite 7[a]



[a]Reagents and conditions: (i) tri(prop-2-ynyl)amine, $[Pd^0[P(Ph_3)_4]$, CuI, dry DMF, Et_3N, rt, 12 h; (ii) *i*-Bu_2O, TMSCl, anhydrous pyridine, rt, 3 h; (iii) 4,4′-dimethoxytriphenylmethyl chloride, anhydrous pyridine, rt, 8 h; (iv) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, anhydrous CH_2Cl_2, $(i\text{-}Pr)_2EtN$, rt, 30 min.

Consequently, compound **1a** has found broad applications as a 2′-deoxyguanosine mimic in nucleoside, nucleotide, and oligonucleotide chemistry and biology. Recent review on pyrrolo-[2,3-*d*]pyrimidines covers various aspects of this molecule.[25]

This paper describes the functionalization of the iodo nucleoside **1b**[26a] with tripropargylamine to yield nucleoside

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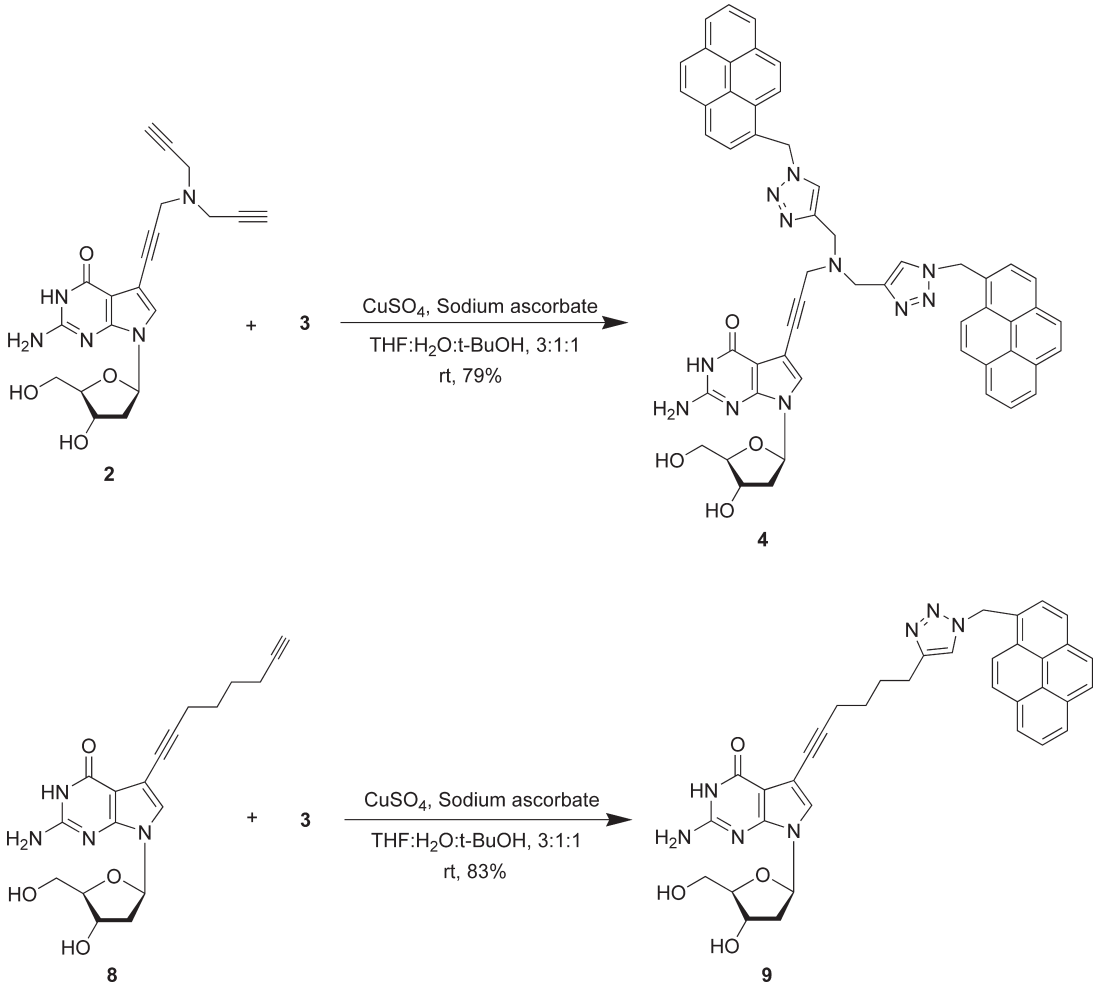
SCHEME 2. Functionalization of Nucleosides 2 and 8 with 1-Azidomethylpyrene (3)



2 followed by the "double click" reaction with the 1-azidomethyl pyrene (**3**), affording the dye conjugate **4**. Oligonucleotides were prepared containing **2**, which were then functionalized in a similar way as the nucleoside. The "double click" protocol is used to introduce identical pyrene labels simultaneously by the azide−alkyne click reaction into a side chain of an oligonucleotide. The influence of the tripropargylamine residue on the duplex stability is investigated, and monomer as well as excimer fluorescence is studied on nucleosides and oligonucleotides. For comparison, pyrene functionalization was also investigated on nucleosides and oligonucleotides bearing an octadiynyl side chain in the 7-position of 7-deaza-2′-deoxyguanosine (**8**).[6d]

Results and Discussion

Synthesis and Characterization of Monomers. For oligonucleotide synthesis, phosphoramidite building block **7** was synthesized. The 7-iodo-7-deaza-2′-deoxyguanosine (**1b**) served as starting material in the Sonogashira cross-coupling reaction. The reaction was performed in dry DMF in the presence of Et_3N, $[Pd^0(PPh_3)_4]$, and CuI, with a 10-fold excess of alkyne affording nucleoside **2** in 55% yield. Next, compound **2** was protected at the 2-amino group with an *iso*-butyryl residue affording the protected intermediate **5** in 72% yield. Then, compound **5** was converted to the 5′-*O*-DMT derivative **6** under standard conditions. Further phosphitylation yielded the phosphoramidite **7** (82%) (Scheme 1).

For the Cu(I)-catalyzed click reaction, the 1-azidomethyl pyrene (**3**) was prepared from 1-pyrenemethanol following an already published procedure.[14a] Next, the "double click" reaction was performed on compound **2** with **3** yielding the bispyrene derivative **4** in 79% yield. We found that both terminal triple bonds were functionalized simultaneously by two reporter groups (pyrenes) even though they are space demanding. Monofunctionalized side chains were not detected, which might result from direct participation of copper ions bound to the tripropargylamine side chain, thereby catalyzing the click reaction at the active center. For comparison, the click reaction was also undertaken on the octa-1,7-diynyl nucleoside **8**[6d] with **3** giving the click product **9** in 83% yield (Scheme 2).

All compounds were characterized by elemental analyses and ^1H and ^{13}C NMR spectra. The ^{13}C NMR chemical shifts are listed in Experimental Section (see also Table S2, Supporting Information). The $^1H-^{13}C$ coupling constants were determined from $^1H-^{13}C$ gated-decoupled spectra (see Table S3, Supporting Information). The intact structure of the tripropargylamine side chain was confirmed by ^{13}C

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TABLE 1. T_m Values and Thermodynamic Data of Oligonucleotide Duplexes Containing the 7-Deazaguanine Derivatives 2 and 8[a]

duplexes	T_m (°C)	ΔT_m[b] (°C)	$\Delta G°_{310}$[c] (kcal/mol)
5′-d(TAG GTC AAT ACT) (**10**)	50	−	−11.8
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TA**2** GTC AAT ACT) (**12**)	52	+2	−13.7
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TAG GTC AAT ACT) (**10**)	50	+0	−12.3
3′-d(ATC CA**2** TTA TGA) (**13**)			
5′-d(TAG GTC AAT ACT) (**10**)	54	+4	−14.4
3′-d(ATC CAG TTA T**2**A) (**14**)			
5′-d(TAG GTC AAT ACT) (**10**)	51	+1	−12.7
3′-d(ATC CA**2** TTA T**2**A) (**15**)			
5′-d(TA**2** **2**TC AAT ACT) (**16**)	54	+4	−14.5
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TA**2** **2**TC AAT ACT) (**16**)	55	+5	−14.3
3′-d(ATC CAG TTA T**2**A) (**14**)			
5′-d(TA**2** **2**TC AAT ACT) (**16**)	56	+6	−14.2
3′-d(ATC CA**2** TTA T**2**A) (**15**)			
5′-d(TA**8** GTC AAT ACT) (**17**)	52	+2	−13.0
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TAG GTC AAT ACT) (**10**)	51	+1	−12.6
3′-d(ATC CA**8** TTA TGA) (**18**)			
5′-d(TA**8** **8**TC AAT ACT) (**19**)	53[6d]	+3	
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TA**8** **8**TC AAT ACT) (**19**)	55[6d]	+5	
3′-d(ATC CAG TTA T**8**A) (**20**)			
5′-d(TA**8** **8**TC AAT ACT) (**19**)	58[6d]	+8	
3′-d(ATC CA**8** TTA T**8**A) (**21**)			

[a]Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration. [b]Refers to the temperature difference of the modified duplex versus the unmodified reference duplex. [c]The $\Delta G°_{310}$ values were calculated with the software package "Cary WinUV" for thermal applications supplied with the Cary UV/vis spectrometer. The $\Delta G°_{310}$ values are given with 15% error.

NMR spectra showing two signals of the methylene groups (41.1 and 42.2 ppm) and four signals for the triple bond carbons (**2**: 75.9, 79.2, 79.3, 84.5 ppm; Table S2), confirmed by inverted signals of distortionless enhancement by polarization transfer (DEPT-135) spectra (Supporting Information). From this, it was concluded that the triple bonds are not affected by the Pd-assisted Sonogashira cross coupling (allene formation[26b]).

Synthesis and Duplex Stability of Oligonucleotides Containing 7-Tripropargylamine Side Chain. Earlier, it was shown that nucleoside derivatives with alkynyl side chains in the 5-position of pyrimidine or the 7-position of pyrrolo-[2,3-*d*]pyrimidine nucleobases are well-accommodated in the major groove of DNA.[6c,d,7,21] In many cases, propynyl and octadiynyl side chains have a positive effect on the stability of the DNA duplex. Recently, a branched tripropargylamine linker was introduced at the 5-position of 2′-deoxyuridine as well as in corresponding oligonucleotides.[8] To evaluate the potential of the 7-tripropargylamine residue on 7-deazapurine bases regarding duplex stability, functionalization, and charge transfer, the "double click" reaction was performed on a series of oligonucleotides. For this, phosphoramidite **7** was employed in solid-phase synthesis in a 1 μmol scale. The coupling yields were always higher than 95%. Deprotection of the oligomers was performed in 25% aqueous NH_3 at 60 °C for 16 h. The oligonucleotides were purified before and after detritylation by reversed-phase HPLC. The incorporation of the modified residue **2** into the oligonucleotides was confirmed by MALDI-TOF mass spectrometry (see Supporting Information, Table S1).

Single and multiple incorporations of **2**, replacing dG residues within various positions of the reference duplex 5′-d(TAG GTC AAT ACT) (**10**) and 3′-d(ATC CAG TTA TGA) (**11**), were performed. Modified oligonucleotides and their duplexes are shown in Table 1. In all cases, more stable duplexes were formed when the tripropargylamine nucleoside **2** was replacing dG. These values are close to the T_m values found for oligonucleotide duplexes containing 7-deaza-7-octa-1,7-diynyl-dG (**8**) at identical positions.[6d] Thus, the more bulky tripropargylamine side chain is not perturbing DNA duplex structure as it was found for the even less space demanding octadiynyl linker. The more hydrophilic character arising by virtue of terminal triple bonds has this advantage over fully saturated side chains. Water molecules will bind to the terminal triple bonds via hydrogen bonding, reducing the hydrophobicity.

Synthesis and Duplex Stability of Oligonucleotide Pyrene Conjugates. Next, the "double click" reaction was performed on the oligonucleotides 5′-d(TA**2** GTC AAT ACT) (**12**) and 5′-d(AGT ATT **2**AC CTA) (**13**) each containing one 7-deaza-7-(tripropargylamine)-2′-deoxyguanosine residue. Both oligonucleotides were functionalized with **3**. The reaction was performed in aqueous solution (H_2O/*t*-BuOH/DMSO) in the presence of $CuSO_4$−TBTA (tris(benzyltriazoylmethyl)amine) (1:1) complex, TCEP (tris(carboxyethyl)-phosphine), and $NaHCO_3$ (Scheme 3). $NaHCO_3$ was essential for the completion of the reaction within 12 h, yielding the strongly fluorescent oligonucleotides 5′-d(TA**4** GTC AAT ACT) (**22**) and 5′-d(AGT ATT **4**AC CTA) (**23**). For comparison, the click reaction was also performed with the pyrene azide **3** on the oligonucleotides 5′-d(TA**8** GTC AAT ACT) (**17**) and 5′-d(AGT ATT **8**AC CTA) (**18**) containing the octadiynyl side chain.

All oligonucleotides were purified by reversed-phase HPLC and characterized by MALDI-TOF mass spectra (see Supporting Information Table S1).

As discussed above, the tripropargylamine as well as the octadiynyl linkers does not perturb the DNA duplex structure. Now, the influence of dye modification on the duplex stability is evaluated. For this, the T_m value of the reference oligonucleotide duplex 5′-d(TAG GTC AAT ACT) (**10**) and 3′-d(ATC CAG TTA TGA) (**11**) modified with **2** and **8**, and functionalized with pyrene azide **3**, was measured. More stable duplexes are formed by the pyrene-modified oligonucleotides compared to the unmodified duplex with a tendency of higher T_m values for modification near the terminus than those modified at central position. Overall, T_m data confirm that even four pyrene ligands do not destabilize the DNA duplex (Table 2).

Fluorescence Properties of Nucleoside and Oligonucleotide Pyrene "Click" Derivatives. Pyrene shows monomer emission around 370−400 nm and excimer fluorescence around 450−500 nm.[17,18] Excimer fluorescence requires a proximal positioning of the pyrene residues.[27] Two[28] or more than two[29] pyrene residues attached to oligonucleotides were used in nucleic acid hybridization, including parallel duplexes,[30]

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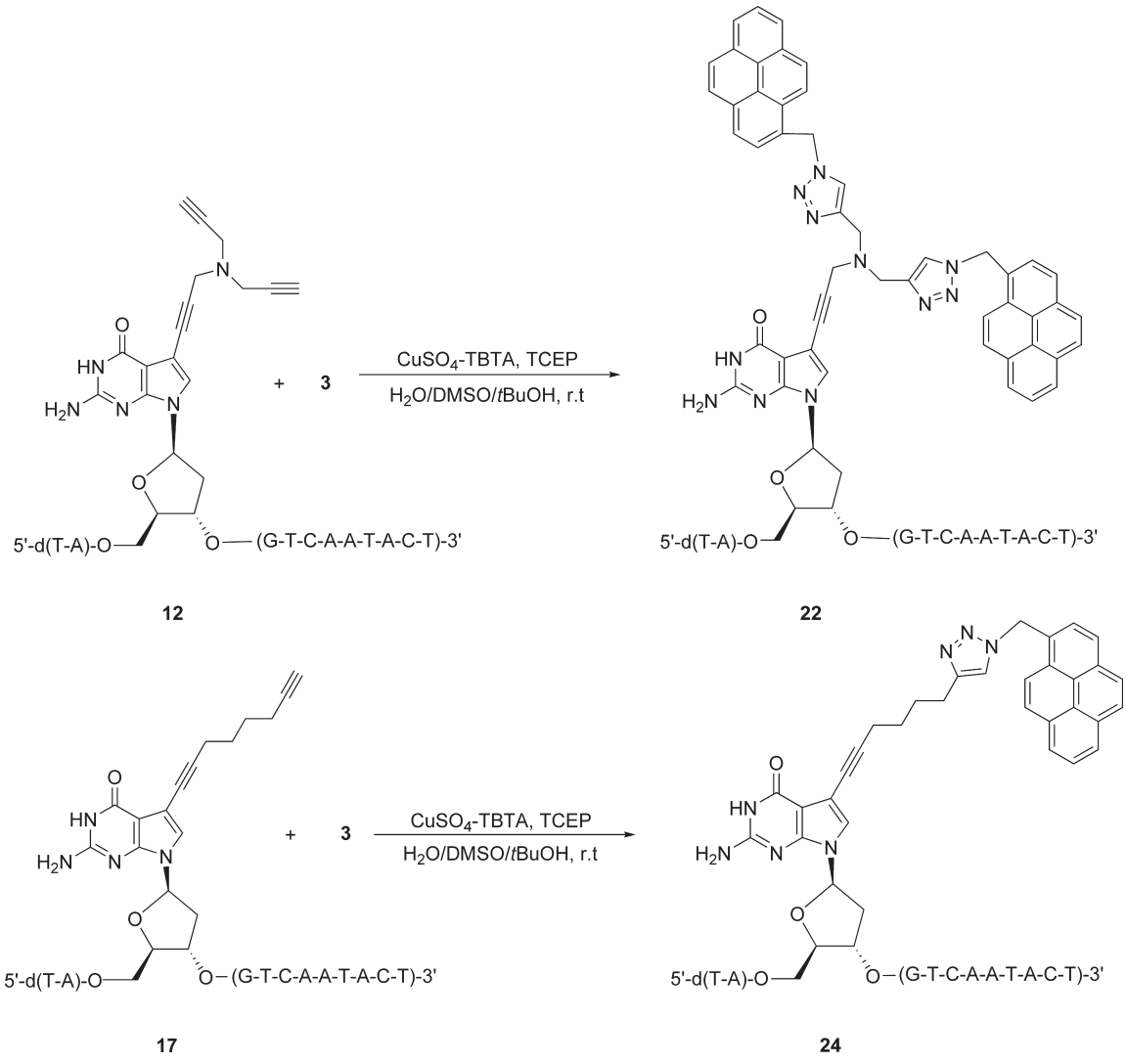
SCHEME 3. Huisgen−Meldal−Sharpless [2 + 3] Cycloaddition of Oligonucleotides 12 and 17 Incorporating Nucleoside 2 and 8 with 1-Azidomethylpyrene



triplexes,[31] and quadruplexes.[32] For this, pyrene residues were linked to various positions of sugar, nucleosides, and oligonucleotides.[9−12] The 2′-hydroxyl group of a sugar moiety was chosen as target position along with the 5-position of pyrimidine and the 8-position of purine bases. Photoexcitable pyrene was used for electron and hole transfer. When the guanine base was modified, charge separation has been observed to form a guanine radical cation and a pyrene radical anion ($Py^{\bullet-}-G^{\bullet+}$).[33] In pyrenyl dU nucleosides, the process is reversed (electron transport). Now, charge separation yields a uracil radical anion and a pyrene radical cation ($Py^{\bullet+}-dU^{\bullet-}$).[34] Consequently, DNA bases act in either way leading to the quenching of pyrene fluorescence. As said earlier, for excimer fluorescence, both pyrene residues have to be in proximal position.[27] Yamana and co-workers exploited this phenomenon by introducing two

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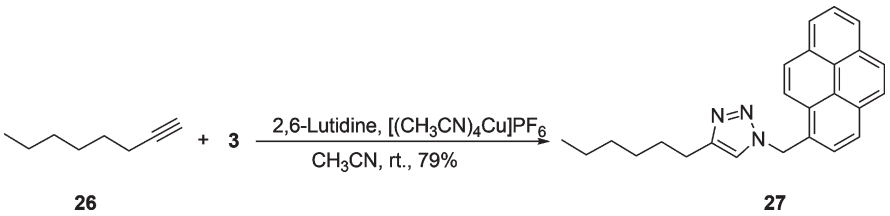
SCHEME 4. Functionalization of 1-Octyne with 1-Azidomethylpyrene



26 **27**

TABLE 2. T_m Values and Thermodynamic Data of Oligonucleotide Duplex Pyrene Conjugates[a]

duplexes	T_m (°C)	ΔT_m[b] (°C)	$\Delta G°_{310}$[c] (kcal/mol)
5′-d(TAG GTC AAT ACT) (**10**)	50	−	−11.8
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TA**4** GTC AAT ACT) (**22**)	54	+4	−13.1
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TAG GTC AAT ACT) (**10**)	51	+1	−11.9
3′-d(ATC CA**4** TTA TGA) (**23**)			
5′-d(TA**9** GTC AAT ACT) (**24**)	54	+4	−13.3
3′-d(ATC CAG TTA TGA) (**11**)			
5′-d(TAG GTC AAT ACT) (**10**)	55	+5	−13.3
3′-d(ATC CA**9** TTA TGA) (**25**)			
5′-d(TA**4** GTC AAT ACT) (**22**)	54	+4	−13.1
3′-d(ATC CA**4** TTA TGA) (**23**)			
5′-d(TA**9** GTC AAT ACT) (**24**)	55	+5	−13.7
3′-d(ATC CA**9** TTA TGA) (**25**)			
5′-d(TA**4** GTC AAT ACT) (**22**)	54	+4	−13.8
3′-d(ATC CA**9** TTA TGA) (**25**)			

[a]Measured at 260 nm in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0) with 5 μM + 5 μM single-strand concentration. [b]Refers to the temperature difference of the modified duplex versus the unmodified reference duplex. [c]The $\Delta G°_{310}$ values were calculated with the software package "Cary WinUV" for thermal applications supplied with the Cary UV/vis spectrometer. The $\Delta G°_{310}$ values are given with 15% error.

pyrene residues in an acyclic propanediol moiety.[35] With this, excimer fluorescence was used to distinguish between single strands and duplex DNA.

To evaluate photophysical properties, UV/vis, excitation, and emission spectra of the nucleoside click products **4** and **9** as well as of oligonucleotide click products **22** and **23** (tripropargylamine pyrene conjugates) and **24** and **25** (octadiynyl pyrene conjugates) were measured. In order to determine the quenching of the pyrene fluorescence by the 7-deazaguanine, the abasic derivative **27** was prepared from 1-octyne (**26**) and the pyrene azide **3** containing all necessary elements of the dye conjugate except 7-deazaguanine (Scheme 4). All measurements were performed in methanol and acetonitrile. For solubility reasons, the monomeric pyrene-1,2,3-triazolyl conjugates **4**, **9**, and **27** were dissolved first in 1 mL of DMSO and then diluted with 99 mL of the solvent (MeOH or MeCN). In all experiments, the concentration of the dye conjugates was identical (6.8×10^{-6} M).

Figure 2a,b shows the UV/vis spectra of pyrene conjugates **4**, **9**, and **27** in methanol and acetonitrile at identical molar concentrations. The tripropargylamine derivative **4** decorated with two pyrene residues shows the highest UV absorbance, while compounds **9** and **27** are less absorbing. No differences were observed regarding the wavelength maxima, retaining the similar absorption pattern.

Next, fluorescence measurements were performed for all three pyrene derivatives (Figure 3) using identical concentrations as used for the measurement of the UV/vis spectra. Only the nucleoside click product **4** with two proximal pyrenes shows strong excimer fluorescence (464 nm) and rather low monomer fluorescence at 377 and 394 nm, while the conjugate **9** containing one pyrene shows only monomeric pyrene emission. Monomeric pyrene emission was also observed for the abasic octyne derivative **27**. From this, we concluded that in conjugate **4** the two pyrenes are in proximal position, thereby developing strong excimer fluorescence in methanol as well as in acetonitrile. For conjugate **4**, the excimer fluorescence was also observed in DMSO/water mixture (data not shown). However, quantification of data was not possible as this conjugate is not fully soluble (opaque solution).

From Figure 3, it is also apparent that both nucleoside conjugates (**4** and **9**) show rather low monomer fluorescence compared to the abasic conjugate **27** (identical concentration were used in all cases). This points to a quenching of the pyrene fluorescence by the 7-deazaguanine moiety within both nucleoside conjugates. As there is no overlap between excitation and emission spectra of the pyrene residue with a nucleobase FRET (Förster resonance energy transfer), quenching results from an intramolecular charge transfer. Upon irradiation, the pyrene-7-deazaguanine conjugate **4** forms a 7-deazaguanine radical cation and a pyrene radical anion ($c^7G^{\bullet+}-Py^{\bullet-}$) resulting in charge separation. A charge transfer was already observed for 7-deazaguanine nucleosides and oligonucleotides with other dyes[36] and for the pyrene residue with canonical bases.[33,34]

Then, the photophysical properties of single-stranded oligonucleotides (ss) and oligonucleotide duplexes (ds) with branched linker were investigated. For that, two oligonucleotides **22** and **23** were selected bearing the two proximal pyrene residues in a central or a flanking position. For comparison, the nonbranched oligonucleotides **24** and **25** with octadiynyl linker located at exactly the same position were measured. Then, the corresponding duplexes were measured. In all cases, the complementary strand was unmodified (**10** and **11**). A 3 nm UV/vis bathochromic shift is observed when duplexes are formed (Figures 4a and 5a). Figures 4b and 5b display the fluorescence emission spectra measured between 360 and 600 nm. Emission maxima are centered near 400 nm. Surprisingly, a pyrene excimer emission is not observed for the ss-oligonucleotides **22** and **23** as well as for their duplexes with two proximal pyrenes in one strand (Figure 4b). Measurements of the octadiynyl oligonucleotides **24** and **25** gave similar results (Figure 5b). Thus, pyrene−pyrene interactions do not exist neither in ss-oligonucleotides nor in corresponding

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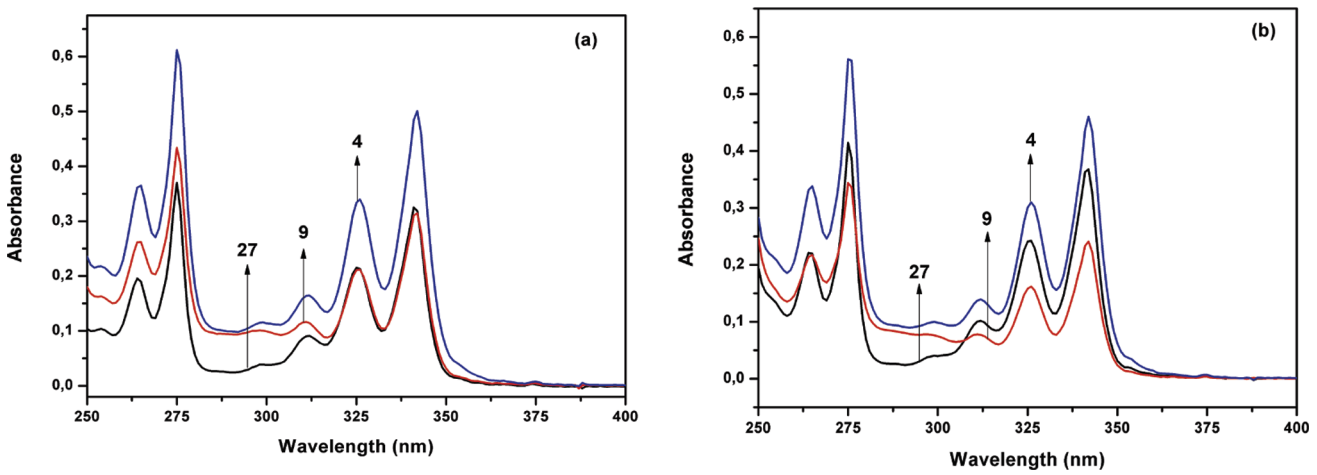

FIGURE 2. UV/vis spectra of nucleoside conjugates **4**, **9**, and **27** in (a) methanol and (b) acetonitrile.

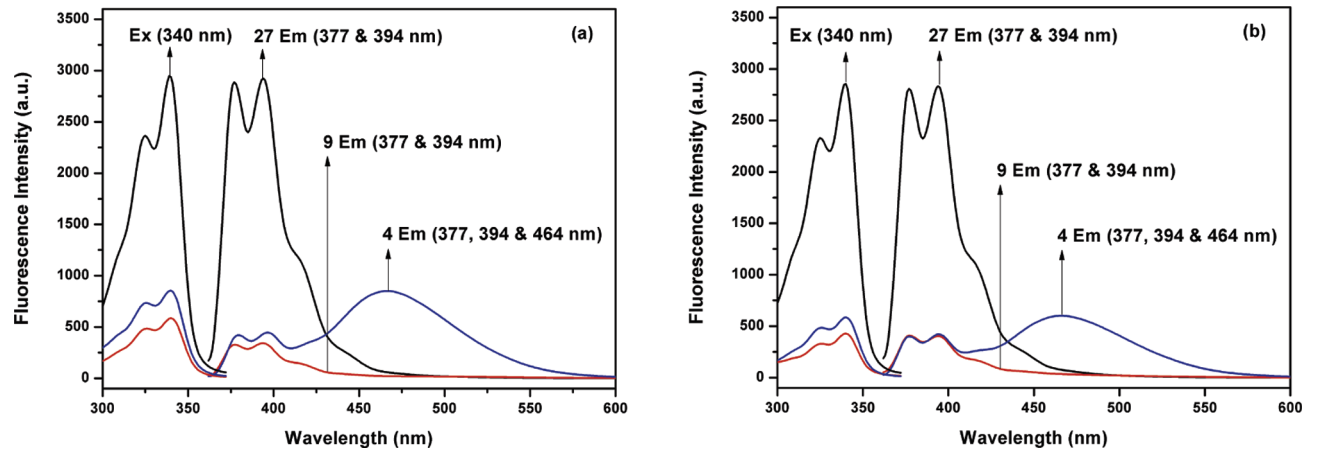

FIGURE 3. Excitation and emission spectra of nucleoside conjugates **4**, **9**, and **27** in (a) methanol and (b) acetonitrile. All nucleoside conjugates were excited at 340 nm.

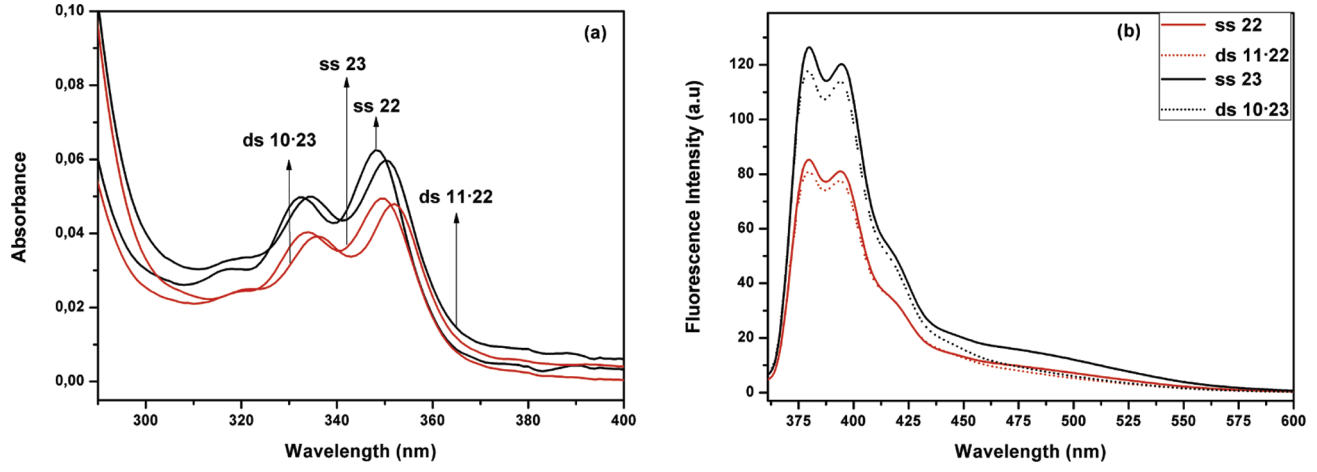

FIGURE 4. (a) UV/vis spectra of the 2 μM single-stranded oligonucleotides of 7-tripropargylamine pyrene conjugates **22**, **23** and duplex DNA **11**·**22**, **10**·**23** (2 μM of each strand). (b) Fluorescence emission spectra of 2 μM single-stranded **22**, **23** and duplex DNA **11**·**22**, **10**·**23** (2 μM of each strand). All spectra were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

duplexes with two proximal pyrenes. This was unexpected as the monomeric tripropargylamine conjugate **4** exhibits strong excimer fluorescence while the octadiynyl conjugate **9** does not (see Figure 3).

Subsequently, UV/vis (Figure 6a) and fluorescence emission spectra (Figure 6b) of duplexes were measured containing pyrene modification in both strands. Oligonucleotides **22**−**25** were selected with either one (octadiynyl derivatives)

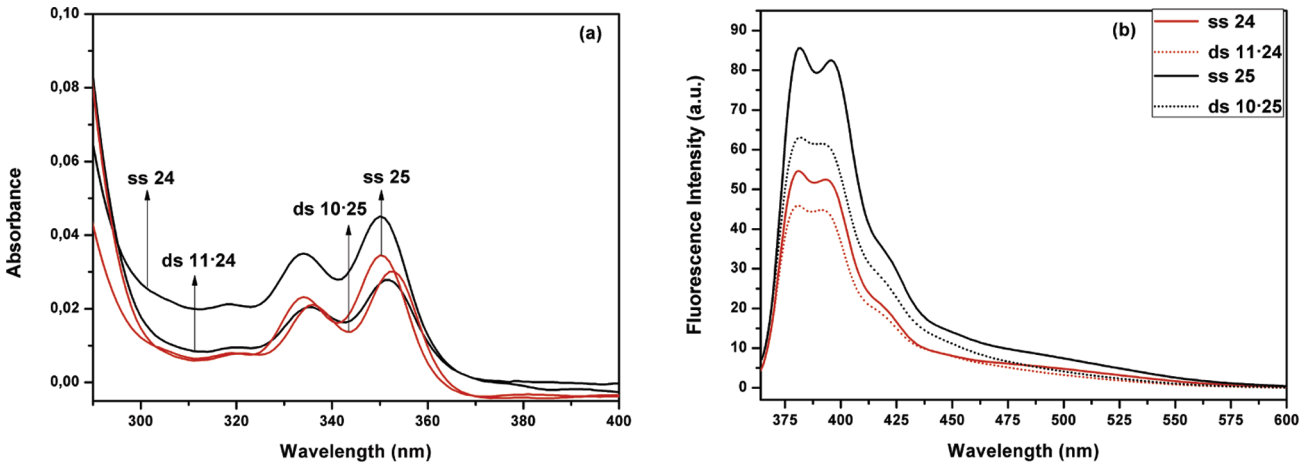

FIGURE 5. (a) UV/vis spectra of the 2 μM single-stranded oligonucleotides of 7-octadiynyl pyrene conjugates **24**, **25** and duplex DNA **11·24**, **10·25**. (b) Fluorescence emission spectra of 2 μM single-stranded oligonucleotides **24**, **25** and duplex DNA **11·24**, **10·25** (2 μM of each strand). All spectra were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

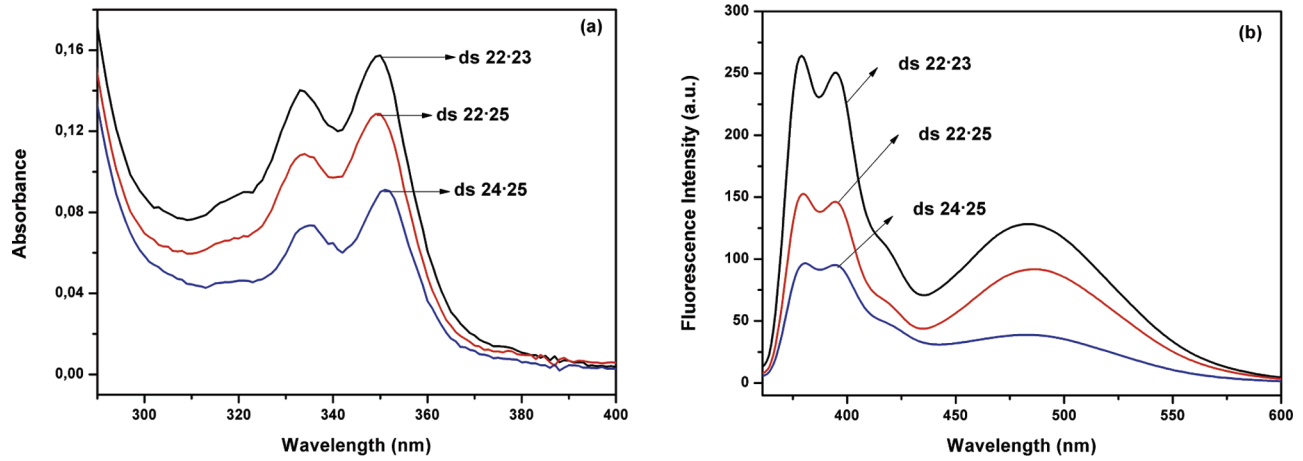

FIGURE 6. (a) UV/vis spectra of duplex **22·23**, **24·25**, and **22·25** (2 μM + 2 μM single-strand concentration). (b) Emission spectra of the duplex **22·23**, **24·25**, and **22·25** (2 μM + 2 μM single-strand concentration) when excited at 341 nm. All spectra were measured in 1 M NaCl, 100 mM $MgCl_2$, and 60 mM Na-cacodylate (pH 7.0).

or two (tripropargylamine derivatives) pyrene residues in each strand. Now, all of the duplex combinations show excimer fluorescence, including those containing only one pyrene modification in each strand. However, the fluorescence intensities are higher for the duplex with four pyrenes (**22·23**) than for those incorporating two or three pyrene residues (Figure 6b). This excimer fluorescence results from an interstrand cross-talk of the pyrene residues. Figure 7 illustrates the set of combination of the various duplexes. However, it does not display the steric consequences. Inspection of a B-DNA model shows that the modification sites separated by two base pairs within the duplex DNA allow a proximal positioning of two pyrene residues within the major groove supplied by complementary strands. Such a phenomenon was already reported for pyrene residues located in the minor groove. In this case, the pyrene moiety was linked to the sugar moiety of locked nucleosides.[37] From Figure 6b, it is obvious that the number of pyrene residues increases the excimer fluorescence.

These findings are supported by molecular dynamics simulations using Amber MM+ force field (Hyperchem 7.0/8.0; Hypercube Inc., Gainesville, FL). Calculations were performed on the 12-mer duplexes **11·22** and **22·23**. The energy-minimized molecular structures are built as B-type DNA and are shown in Figure 8. Figure 8a displays a duplex decorated with two pyrene moieties (**11·22**) in which a terminal dG residue is replaced by the branched tripropargylamine derivative of 7-deaza-2′-deoxyguanosine. Moreover, the duplex structure is not disturbed even after modification. As indicated in the picture, the pyrene residues lie apart from each other, exhibiting only monomer fluorescence. A duplex containing two 7-tripropargylamine-7-deaza-2′-deoxyguanosine functionalized with pyrene residues (**22·23**), one in each strand, displays an excimer fluorescence (Figure 8b). The two pyrene residues in this duplex lie in a close proximity facilitating the $\pi-\pi$ interaction between electronic clouds, thus giving rise to excimer fluorescence.[27] It is noteworthy that each pyrene residue involved in excimer formation comes from opposite strands.

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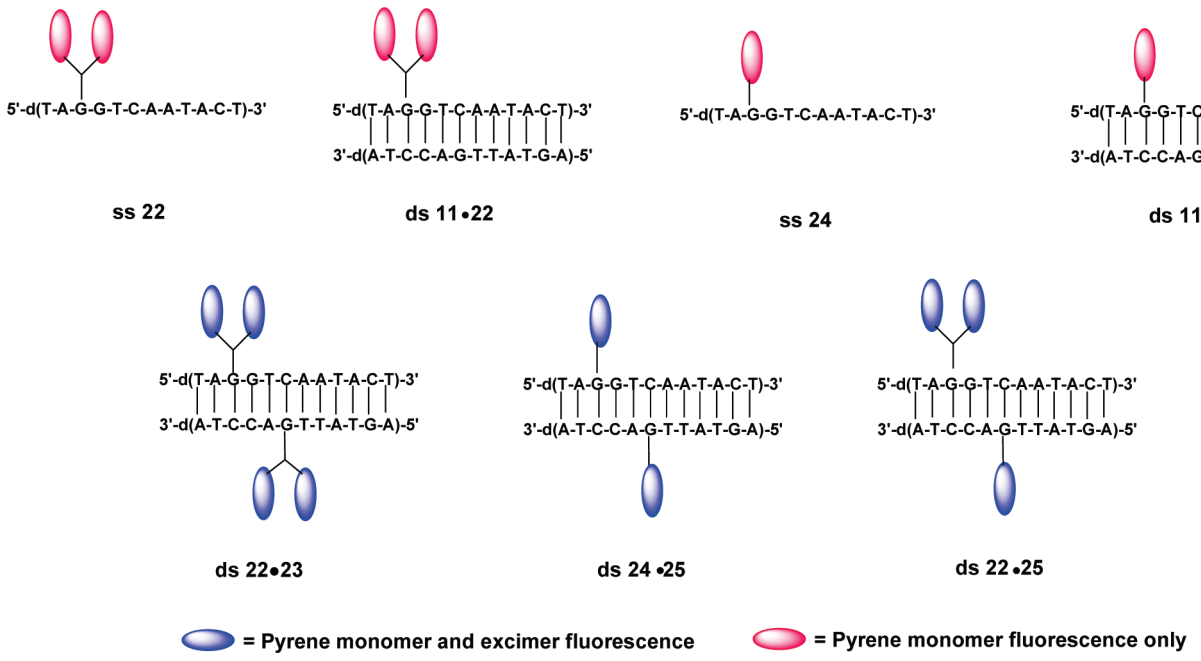

FIGURE 7. Pyrene monomer and excimer fluorescence of ss- and ds-oligonucleotides.

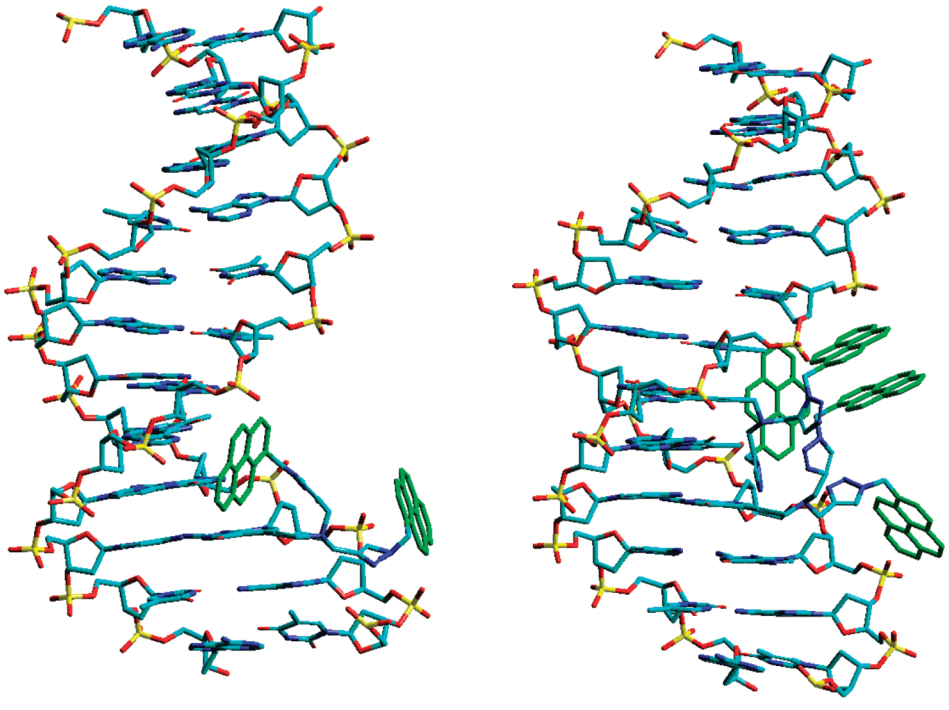

FIGURE 8. Molecular models of (a) duplex 5′-d(TA**4** GTC AATACT) (**22**)•3′-d(ATC CAG TTA TGA) (**11**) and (b) duplex 5′-d(TA**4** GTC AAT ACT) (**22**)•3′-d(ATC CA**4** TTA TGA) (**23**). The models were constructed using Hyperchem 7.0/8.0 and energy minimized using AMBER calculations.

Conclusion

The 7-tripropargylamine-7-deazaguanine nucleoside (**2**) was synthesized by the Sonogashira cross-coupling reaction with branched side chains containing two terminal triple bonds. For this, 7-iodo-7-deaza-2′-deoxyguanosine (**1b**) was converted into the 7-tripropargylamine derivative **2** and corresponding phosphoramidite building block **7** was synthesized. Oligonucleotides incorporating **2** were prepared and hybridized. Modified duplexes with single or multiple incorporations have a positive effect on the DNA duplex stability and do not perturb the DNA structure. Cu(I)-assisted functionalization of both terminal triple bonds by

the Huisgen−Meldal−Sharpless click reaction, "double click reaction", with the pyrene azide **3** results in nucleosides and oligonucleotides with proximal pyrenes. The space demanding reporter groups are well-accommodated in the major groove of DNA due to the noncritical modification site (N-7) of the 7-deazaguanine base. While the monomeric tripropargylamine click product **4** with two proximal pyrenes shows excimer fluorescence, the octadiynyl click adduct **9** with one pyrene unit does not. The ss-oligonucleotides containing compound **4** as well as **9** do not show excimer fluorescence. The same is observed for ds-oligonucleotides with unmodified complementary strands. However, duplex DNA bearing pyrene residues in both strands exhibits strong excimer fluorescence when each strand contains a tripropargyl moiety clicked with two pyrenes each. The DNA duplex having two pyrene reporter groups in each strand displays weak excimer fluorescence when the octadiynyl side chain is functionalized with **3**. This pyrene−pyrene interstrand interaction occurs when the pyrene modification sites are separated by two base pairs, which bring the fluorescent dyes in a proximal position. However, the linear increase in the excimer fluorescence intensity with a number of pyrene units may account for both the number of pyrene residues as well as their most favored spatial alignment, which brings them to interactable proximity. Molecular dynamics calculations show that only two out of four pyrene residues are communicating, thereby forming the exciplex, while the other two are buried in the major groove of DNA. The modeling data are supported by the photophysical properties of the pyrene-modified duplexes showing monomer and excimer fluorescence. Thus, hybridization can be detected by pyrene excimer fluorescence when a click reaction is performed on oligonucleotides containing triprogargylamine as well as octadiynyl side chains.

Experimental Section

2-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-3,7-dihydro-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one (2). To a suspension of **1b** (1.0 g, 2.55 mmol) and CuI (0.097 g, 0.51 mmol) in anhydrous DMF (10 mL) were added successively $[Pd(PPh_3)_4]$ (0.295 g, 0.255 mmol), anhydrous Et_3N (0.516 g, 5.1 mmol), and tri(prop-2-ynyl)amine (3.345 g, 25.5 mmol). The reaction was stirred at room temperature under nitrogen atmosphere and allowed to proceed until the starting material was consumed (TLC monitoring). Then, the mixture was diluted with MeOH−CH_2Cl_2 (1:1, 25 mL), and Dowex HCO_3^- form (100−200 mesh, 1.5 g) was added. After stirring for 15 min, the evolution of gas ceased. Stirring was continued for another 30 min, and the resin was filtered off and washed with MeOH−CH_2Cl_2 (1:1, 250 mL). The combined filtrate was concentrated and the residue purified by flash chromatography (FC) (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 96:4) to give **2** (0.554 g, 55%) as a colorless solid: TLC (CH_2Cl_2/MeOH 90:10) R_f 0.23; λ_{max} (MeOH)/nm 239 (ε/dm^3 mol^{-1} cm^{-1} 26 600), 273 (13 200), 291 (11 500); ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm) 2.04−2.11 (m, 1H, H_α-2′), 2.27−2.36 (m, 1H, H_β-2′), 3.21−3.32 (m, 2H, 2 × C≡CH), 3.46−3.52 (m, 6H, 3 × NCH_2), 3.55 (s, 2H, H-5′), 3.74−3.77 (m, 1H, H-4′), 4.27−4.28 (m, 1H, H-3′), 4.90−4.94 (t, J = 5.7 Hz, 1H, HO-5′), 5.21−5.23 (d, J = 3.6 Hz, 1H, HO-3′), 6.24−6.29 (m, 1H, H-1′), 6.36 (s, 2H, NH_2), 7.25 (s, 1H, H-8), 10.42 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm) 157.9, 153.2, 150.3, 121.7, 99.5, 98.3, 87.2, 84.5, 82.3, 79.3, 79.2, 75.9, 70.9, 61.9, 42.2, 41.1. Anal. Calcd for $C_{20}H_{21}N_5O_4$ (395.41): C, 60.75; H, 5.35; N, 17.71. Found: C, 60.63; H, 5.26; N, 17.60.

7-(2-Deoxy-β-D-*erythro*-pentofuranosyl)-2-(isobutyrylamino)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-3,7-dihydro-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one (5). Compound **2** (0.3 g, 0.76 mmol) was dried by repeated coevaporation with anhydrous pyridine (3 × 5 mL) and then dissolved in anhydrous pyridine (8 mL), and trimethylsilyl chloride (0.412 g, 3.79 mmol) was added to the solution. The reaction mixture was stirred for 15 min at room temperature, and then isobutyric anhydride (0.6 g, 3.79 mmol) was added. The solution was stirred for 3 h at room temperature. Then, the solution was cooled in an ice bath, and H_2O (1 mL) and subsequently (5 min later) 28−30% aq NH_3 solution (1 mL) were added, and stirring was continued for 30 min at room temperature. The solvent was evaporated to near dryness, coevaporated with toluene (3 × 5 mL), and the residue was purified by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/MeOH 97:3) to give **5** (0.255 g, 72%) as a colorless solid: TLC (CH_2Cl_2/MeOH 90:10) R_f 0.51; λ_{max} (MeOH)/nm 238 (ε/dm^3 mol^{-1} cm^{-1} 19 500), 281 (16 100); ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm) 1.10, 1.12 (s, 6H, 2 × CH_3), 2.10−2.16 (m, 1H, H_α-2′), 2.33−2.42 (m, 1H, H_β-2′), 2.67−2.78 (m, 1H, CH), 3.22 (s, 2H, 2 × C≡CH), 3.46−3.53 (m, 6H, 3 × NCH_2), 3.58 (s, 2H, H-5′), 3.78−3.80 (d, J = 1.8 Hz, 1H, H-4′), 4.30−4.31 (d, J = 2.4 Hz, 1H, H-3′), 4.93−4.96 (t, J = 5.1 Hz, 1H, HO-5′), 5.25−5.26 (d, J = 3.33 Hz, 1H, HO-3′), 6.34−6.39 (m, 1H, H-1′), 7.56 (s, 1H, H-8), 11.57, 11.80 (s, 2H, 2 × NH); ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm) 180.0, 155.9, 147.6, 147.1, 123.9, 103.5, 98.9, 87.4, 85.4, 82.7, 79.2, 78.3, 75.9, 70.8, 61.7, 42.1, 41.1, 34.8, 18.9, 18.9. Anal. Calcd for $C_{24}H_{27}N_5O_5$ (465.50): C, 61.92; H, 5.85; N, 15.04. Found: C, 61.91; H, 5.82; N, 15.00.

7-(2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)-2-(isobutyrylamino)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-3,7-dihydro-4*H*- pyrrolo-[2,3-*d*]pyrimidin-4-one (6). Compound **5** (0.300 g, 0.65 mmol) was dried by repeated coevaporation with anhydrous pyridine (3 × 5 mL). The residue was dissolved in anhydrous pyridine (8 mL) and stirred with 4,4′-dimethoxytrityl chloride (0.338 g, 0.97 mmol) at room temperature for 8 h. The solution was poured into 5% aq $NaHCO_3$ solution and extracted with CH_2Cl_2 (3 × 30 mL). The combined extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was purified by FC (silica gel, column 15 × 3 cm, CH_2Cl_2/acetone 88:12) to give **6** (0.361 g, 73%) as a colorless solid: TLC (CH_2Cl_2/acetone 80:20) R_f 0.40; λ_{max} (MeOH)/nm 236 (ε/dm^3 mol^{-1} cm^{-1} 35 600), 281 (17 300); ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm) 1.10, 1.13 (s, 6H, 2 × CH_3), 2.24−2.26 (m, 1H, H_α-2′), 2.47−2.55 (m, 1H, H_β-2′), 2.71−2.80 (m, 1H, CH), 3.05−3.18 (m, 2H, H-5′), 3.22 (s, 2H, 2 × C≡CH), 3.45 (s, 4H, 2 × NCH_2), 3.57 (s, 2H, NCH_2), 3.72 (s, 6H, 2 × OCH_3), 3.91−3.92 (m, 1H, H-4′), 4.33 (s, 1H, H-3′), 5.32−5.33 (d, J = 3.9 Hz, 1H, HO-3′), 6.36−6.41 (t, J = 6.3 Hz, 1H, H-1′), 6.82−6.85 (d, J = 8.4 Hz, 2H, Ar−H), 7.21−7.37 (m, 9H, Ar−H), 7.42 (s, 1H, H-8), 11.59, 11.84 (s, 2H, 2 × NH); ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm) 179.6, 157.5, 155.4, 147.1, 146.8, 144.4, 135.1, 134.9, 129.2, 127.3, 127.2, 126.1, 123.3, 112.6, 103.3, 98.5, 85.1, 85.0, 82.2, 78.7, 77.6, 75.3, 70.0, 63.6, 54.5, 41.6, 40.6, 34.3, 29.9, 18.4, 18.3. Anal. Calcd for $C_{45}H_{45}N_5O_7$ (767.87): C, 70.39; H, 5.91; N, 9.12. Found: C, 70.36; H, 6.01; N, 9.06.

7-[2-Deoxy-5-*O*-(4,4′-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl]-2-(isobutyrylamino)-5-{3-[di(prop-2-ynyl)amino]prop-1-ynyl}-3,7-dihydro-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one-3′-[(2-cyanoethyl)-*N*,*N*-(diisopropyl)]phosphoramidite (7). To stirred solution of **6** (0.2 g, 0.26 mmol) in anhydrous CH_2Cl_2 (10 mL) was preflushed with nitrogen and treated with (*i*-Pr)$_2$NEt (0.067 g, 0.52 mmol) followed by 2-cyanoethyl-*N*,*N*-diisopropylphosphoramidochloridite (0.082 g, 0.35 mmol). After stirring for 30 min at room temperature, the solution was diluted with CH_2Cl_2 (30 mL) and extracted with 5% aquous $NaHCO_3$ solution (20 mL). The organic layer was dried over Na_2SO_4 and evaporated. The residue was purified by FC (silica gel, column 10 × 2 cm, CH_2Cl_2/acetone 90:10) to give **7** (0.206 g, 82%) as a colorless foam: TLC (CH_2Cl_2/acetone 90:10) R_f 0.26; ^{31}P NMR ($CDCl_3$) δ 147.4, 148.1.

Huisgen−Meldal−Sharpless [3 + 2] Cycloaddition of Compound 2 with 3. 2-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,7-dihydro-5-{[di(1′,2′,3′-triazol-1-methylpyrene]-propargylamino}-4*H*-pyrrolo-[2,3-*d*]pyrimidin-4-one (4). Compound **2** (0.06 g, 0.15 mmol) and 1-azidomethyl pyrene (0.104 g, 0.4 mmol) were dissolved in THF/H_2O/*t*BuOH (3:1:1, v/v, 4 mL), then sodium ascorbate (60 μL, 0.06 mmol) of freshly prepared 1 M solution in water was added, followed by the addition of copper-(II) sulfate pentahydrate 7.5% in water (54 μL, 0.015 mmol). The reaction mixture was stirred for 16 h at room temperature. The solvent was evaporated, and the residue was purified by FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 90:10) to give **4** (0.110 g, 79%) as a colorless solid: TLC (CH_2Cl_2/MeOH 90:10) R_f 0.53; λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 54 100), 275 (90 720), 312 (24 200), 326 (49 900), 342 (73 600); ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm) 2.03−2.09 (m, 1H, H_α-2′), 2.26−2.35 (m, 1H, H_β-2′), 3.33 (s, 2H, NCH_2), 3.46−3.49 (m, 2H, H-5′), 3.74 (s, 5H, 2 × NCH_2, H-4′), 4.27 (s, 1H, H-3′), 4.89−4.93 (t, J = 5.1 Hz, 1H, HO-5′), 5.22−5.23 (d, J = 3.3 Hz, 1H, HO-3′), 6.25−6.38 (m, 7H, H-1′, 2 × pyrene-CH_2, NH_2), 7.24 (s, 1H, H-8), 7.95−8.33 (m, 18H, Ar−H), 8.50, 8.53 (s, 2H, 2 × H-5-triazole), 10.45 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm) 158.1, 153.2, 150.4, 143.8, 130.9, 130.7, 130.1, 129.3, 128.3, 128.2, 127.7, 127.6, 126.4, 126.1, 125.6, 125.0, 124.6, 124.0, 123.7, 122.8, 121.7, 99.7, 98.5, 87.2, 84.7, 82.3, 79.6, 70.9, 61.9, 50.8, 47.5, 42.4, 30.7. Anal. Calcd for $C_{54}H_{43}N_{11}O_4$ (909.99): C, 71.27; H, 4.76; N, 16.93. Found: C, 70.91; H, 4.39; N, 16.52.

Huisgen−Meldal−Sharpless [3 + 2] Cycloaddition of Compound 8 with 3. 2-Amino-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)-3,7-dihydro-5-{[1′,2′,3′-triazol-1-methylpyrene]hexylidyne}-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one (9). As described for **4**, compound **8** (0.06 g, 0.16 mmol), 1-azidomethyl pyrene (0.058 g, 0.23 mmol), THF/H_2O/*t*BuOH (3:1:1, v/v, 4 mL), sodium ascorbate (67 μL, 0.064 mmol), copper(II) sulfate pentahydrate (53 μL, 0.016 mmol), and FC (silica gel, column 10 × 3 cm, CH_2Cl_2/MeOH 90:10) gave **9** (0.085 g, 83%) as a colorless solid: TLC (CH_2Cl_2/MeOH 90:10) R_f 0.43; λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 39 400), 275 (64 700), 312 (17 400), 326 (31 600), 342 (47 300); ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm) 1.47−1.52 (m, 1H, CH_2), 1.67−1.72 (m, 1H, CH_2), 2.02−2.08 (m, 1H, H_α-2′), 2.27−2.37 (m, 3H, H_β-2′, CH_2), 2.58−2.63 (t, J = 7.2 Hz, 2H, CH_2), 3.47−3.53 (m, 2H, H-5′), 3.75 (s, 1H, H-4'), 4.27 (s, 1H, H-3′), 4.91−4.94 (t, J = 5.4 Hz, 1H, HO-5′), 5.22−5.23 (d, J = 3.6 Hz, 1H, HO-3′), 6.26−6.32 (m, 5H, H-1′, pyrene-CH_2, NH_2), 7.13 (s, 1H, H-8), 7.96−8.34 (m, 9H, Ar−H), 8.49, 8.52 (s, 2H, 2 × H-5-triazole), 10.42 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm) 157.9, 153.1, 150.2, 147.0, 131.0, 130.7, 130.2, 129.3, 128.4, 128.2, 127.7, 127.5, 127.3, 126.5, 125.7, 125.5, 125.0, 124.0, 123.9, 123.7, 122.8, 122.2, 121.1, 99.5, 99.5, 89.7, 87.1, 82.2, 74.6, 70.9, 61.9, 50.7, 28.1, 27.9, 24.5, 18.8. Anal. Calcd for $C_{36}H_{33}N_7O_4$ (627.69): C, 68.88; H, 5.30. Found: C, 69.28; H, 5.64.

Huisgen−Meldal−Sharpless [3 + 2] Cycloaddition of Compound 26 with 3. 4-Hexyl-1-methylpyrene-1*H*-[1,2,3]-triazole (27). 1-Octyne (0.042 g, 0.38 mmol) in acetonitrile (2 mL) was treated sequentially with 1-azidomethyl pyrene (0.146 g, 0.57 mmol), 2,6-lutidine (0.041 g, 0.38 mmol), and $[(CH_3CN)_4Cu]PF_6$ (1.3 mol % with respect to total alkyne units). Then, the reaction mixture was stirred at room temperature for 24 h, and a white solid precipitated from the reaction mixture. Filtration and washing with cold acetonitrile afforded **27** as a colorless solid (0.110 g, 79%): TLC (PE/EtOAc 80:20) R_f 0.26; λ_{max} (MeOH)/nm 265 (ε/dm^3 mol^{-1} cm^{-1} 28 900), 275 (54 400), 312 (13 400), 326 (31 700), 342 (48 100); ^{1}H NMR ($CDCl_3$, 300 MHz) (δ, ppm) 0.78 (s, 3H, CH_3), 1.20 (s, 6H, 3 × CH_2), 1.50−1.55 (m, 2H, CH_2), 2.56−2.61 (t, J = 7.5 Hz, 2H, CH_2), 6.21 (s, 2H, pyrene-CH_2), 7.00 (s, 1H, H-5-triazole), 7.92−8.23 (m, 9H, Ar−H); ^{13}C NMR ($CDCl_3$, 75 MHz) (δ, ppm) 148.9, 132.0, 131.2, 130.6, 129.3, 128.9, 128.2, 125.5, 127.2, 127.2, 126.4, 125.9, 125.0, 124.9, 124.5, 122.0, 120.4, 52.3, 31.5, 29.3, 28.8, 25.7, 22.5, 14.0. Anal. Calcd for $C_{25}H_{25}N_3$ (367.49): C, 81.71; H, 6.86; N, 11.43. Found: C, 81.70; H, 7.00; N, 11.35.

General Procedure for Huisgen−Meldal−Sharpless [3 + 2] Cycloaddition Performed on Oligonucleotides in Aqueous Solution with 1-Azidomethylpyrene 3. To a ss-oligonucleotide (5 A_{260} units) were added $CuSO_4$·TBTA (1:1) ligand complex (50 μL of a 20 mM stock solution in *t*-BuOH/H_2O 1:9), tris(carboxyethyl)-phosphine (TCEP, 50 μL of a 20 mM stock solution in water), $NaHCO_3$ (50 μL, 20 mM stock solution in water), 1-azidomethyl-pyrene (**3**, 100 μL of a 20 mM stock solution in H_2O/dioxane/DMSO, 1:1:1), and DMSO (30 μL), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was concentrated in a speed vac and dissolved in 500 μL bidistilled water and centrifuged for 30 min at 14 000 rpm. The supernatant solution was collected and further purified by reversed-phase HPLC with the gradient 0−20 min 0−20% B in A, 20−25 min 20% B in A, 25−30 min 20−0% B in A, flow rate 1 cm^3 min^{-1}. The molecular masses of the oligonucleotides were determined by MALDI-TOF spectra (Table S1, Supporting Information).

Acknowledgment. We thank Mr. N. Q. Tran for the oligonucleotide synthesis, and Dr. R. Thiele from Roche Diagnostics, Penzberg, for the measurement of the MALDI spectra. We thank Dr. S. Budow for her continuous support throughout the preparation of the manuscript and appreciate critical reading of the manuscript by Dr. P. Leonard and Mr. S. Pujari. Financial support by the Roche Diagnostics GmbH, Penzberg, and ChemBiotech, Münster, Germany, is gratefully acknowledged.

Supporting Information Available: Synthesis, purification, and characterization of oligonucleotides **10**−**25**; molecular masses ([M − H]$^-$) of oligonucleotides **12**−**18** and **22**−**25** measured by MALDI-TOF mass spectrometry (Table S1); HPLC profiles of oligonucleotides and oligonucleotide pyrene conjugates **12**, **14**, **16**, **18**, **22**, and **25** (Figures S1 and S2); ^{13}C NMR chemical shifts (Table S2) and ^{1}H−^{13}C coupling constants of 7-deaza-2′-deoxyguanosine derivatives (Table S3); ^{1}H NMR, ^{13}C NMR, DEPT-135, and ^{1}H−^{13}C gated-decoupled spectra of new compounds (Figure S3−S26); and ^{31}P NMR spectrum of phosphoramidite **7** (Figure S27). This material is available free of charge via the Internet at http://pubs.acs.org.